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Impact of age and antibody type on progression from single to multiple autoantibodies in type 1 diabetes relatives

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Abstract:	Context Islet autoantibodies are markers of type 1 diabetes and an increase in number of autoantibodies detected during the preclinical phase is predictive of progression to overt disease. Objective To refine the impact of age in relation to islet antibody type on the progression from single to multiple autoantibodies in relatives of people with type 1 diabetes. Research design and methods We examined 994 relatives with normal glucose tolerance and positive for a single autoantibody, followed prospectively in the TrialNet Pathway to Prevention. Antibodies to GAD (GADA), insulin (IAA), IA-2 (IA-2A), zinc transporter 8 (ZnT8A) and ICA were tested every 6-12 months. The primary outcome was confirmed development of multiple autoantibodies. Age was categorized as <8yr; 8-11yr; 12-17yr; ≥18yr and optimal age breakpoints identified by recursive partitioning analysis. Results

	After median follow-up of 2 years, 141 relatives had developed ≥ 1 additional autoantibodies. Five-year risk was inversely related to age, but the pattern differed by antibody type: relatives with GADA showed a gradual decrease in risk over the four age groups, while relatives with IAA showed a sharp decrease above the age of 8 years. Recursive partitioning analysis identified age breakpoints at 14 years in relatives with GADA and at 4 years in relatives with IAA. Conclusions In relatives with IAA, spread of islet autoimmunity is largely limited to early childhood, while immune responses initially directed at GAD can mature over a longer period of time. These differences have important implications for monitoring these subjects and for designing prevention trials.
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Impact of age and antibody type on progression from single to multiple autoantibodies in type 1 diabetes relatives (jc.2017-00569).

Dear Editor,

In response to the comments of reviewers, please find hereafter our comments:

Reviewer #1:

In addition to the 994 relatives positive for a single autoantibody (GADA, IA-2A or IAA) with normal oral glucose tolerance at baseline:

State how many of the 151,458 relatives screened in TrialNet PTP between March 1st 2004 and March 31st 2015 were:

1) single autoantibody-positive with dysglycaemia at baseline;
2) multiple autoantibody-positive with normoglycaemia and dysglycaemia, respectively.

Response: We appreciate the reviewer's request for clarification related to how this cohort compares to those who were not included. Of all subjects who were confirmed as positive for a single autoantibody, 994 were identified as normoglycaemic (presented in this manuscript) and an additional 276 subjects had abnormal glucose tolerance. We have included these clarifications in the text of the paper (line 136-138).

We have specifically not included information related to those subjects who were positive for multiple autoantibodies; that cohort is being described through another manuscript.

State how many of the 994 relatives were at baseline ZnT8A positive and negative, respectively, and consider this status in the analysis and interpretation.

Response: As reported in the methods section (lines 102-104), participants included in this study were those screened and confirmed positive for only one autoantibody (GADA, IAA or IA-2A) and were also additionally tested for islet cell antibodies (ICA) and ZnT8A . Therefore, all 994 relatives included in these analyses were ZnT8A-negative, since any subjects who tested positive for ZnT8A (or any other additional autoantibody) were classified as multiple antibody-positive.

Clarify the primary outcome: "… confirmed development of multiple autoantibodies defined as detection on two (subsequent?) occasions …"

Response: We again appreciate the request for clarification on this important point. Indeed, the primary endpoint for this paper is confirmed development of multiple autoantibodies, which we have defined as being identified as having two or more positive autoantibodies (GADA, IAA, IA-2A, ZnT8A, or ICA). This positivity is required to be confirmed on two consecutive evaluations that are done within one year of each other. The date the subject was identified as confirmed positive for multiple autoantibodies is the date of the first multiple antibody positive result that was subsequently confirmed. This has been clarified in the text (lines 120-121).

Importantly, the authors should include HLA in their analysis.

Response: Analyses of the associations between HLA, autoantibody type and risk of progression in this group of confirmed single antibody-positive relatives have already been published in Bingley PJ et al. Diabetologia 2016;59:542-549 (ref 16). We do not therefore feel it is appropriate to add it to the present report. We have however highlighted the previous analysis in the discussion (line 183-185).

Reviewer #2:

It would be helpful briefly to describe the technique of recursive partitioning analysis.

Response: We thank the reviewer for this helpful suggestion, and have included some clarification on this in the statistical methods section (lines 128-130). Overall, recursive partitioning is a model-based and iterative approach that can be used to identify cutpoints of a marker that best differentiate prognosis based on the outcome of interest, here, risk of progression to multiple positive autoantibodies.

In Figures 1 and 2 the decimal points on the y-axis are unnecessary.

Response: We acknowledge that decimal points are not necessarily required in these time-to-event Kaplan-Meier plots for the distributions of time to progression to multiple positive autoantibodies. However, their inclusion is not erroneous. Nonetheless, we modified the formatting of the y-axis for Figures 2a and 2b to make it more readable.

Perhaps add a rider that we do not know if this information necessarily holds true for older people developing type 1 diabetes in their thirties or forties.

Response: We acknowledge that many of these subjects included in our analysis are younger. However, the age range for subjects in these analyses is from 1 to 51 years old at positive autoantibody determination. In fact, 375 of these subjects were 30 years or older. While we did not conduct a subset analysis focusing on these older subjects, they are indeed well represented in this cohort.

Full title

Impact of age and antibody type on progression from single to multiple autoantibodies in type 1 diabetes relatives

Short title

Autoantibody progression in pre-type 1 diabetes

Authors

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* A complete list of the Type 1 Diabetes TrialNet Study Group can be found in the Supplementary Data online

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Duality of interest

No dualities of interest relevant to this article are reported by the Authors.

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Context. Islet autoantibodies are markers of type 1 diabetes and an increase in number of autoantibodies detected during the preclinical phase is predictive of progression to overt disease.

Objective. To refine the impact of age in relation to islet antibody type on the progression from single to multiple autoantibodies in relatives of people with type 1 diabetes.

Research design and methods. We examined 994 relatives with normal glucose tolerance and positive for a single autoantibody, followed prospectively in the TrialNet Pathway to Prevention. Antibodies to GAD (GADA), insulin (IAA), IA-2 (IA-2A), zinc transporter 8 (ZnT8A) and ICA were tested every 6-12 months. The primary outcome was confirmed development of multiple autoantibodies. Age was categorized as <8yr; 8-11yr; 12-17yr; ≥18yr and optimal age breakpoints identified by recursive partitioning analysis.

Results. After median follow-up of 2 years, 141 relatives had developed ≥ 1 additional autoantibodies. Five-year risk was inversely related to age, but the pattern differed by antibody type: relatives with GADA showed a gradual decrease in risk over the four age groups, while relatives with IAA showed a sharp decrease above the age of 8 years. Recursive partitioning analysis identified age breakpoints at 14 years in relatives with GADA and at 4 years in relatives with IAA.

Conclusions. In relatives with IAA, spread of islet autoimmunity is largely limited to early childhood, while immune responses initially directed at GAD can mature over a longer period of time. These differences have important implications for monitoring these subjects and for designing prevention trials.

Introduction

Islet autoimmunity leading to type 1 diabetes develops and progresses silently over many years before glucose intolerance and symptomatic hyperglycemia occur (1). Islet autoantibodies are the best validated markers of this ongoing pathogenetic process and are used to predict clinical disease (2) and stage its preclinical phase (3).

Several prospective studies, including those following infants at genetic risk from birth, have shown that the antibody response against β-cells within pancreatic islets usually targets several autoantigens including insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma-associated antigen 2/ICA512 (IA2A) and zinc transporter 8 (ZnT8A), in varying sequence. Maturation of this humoral immune response, as shown by increasing autoantibody number, titer and affinity, is associated with an increased risk of progression to the disease (4).

Specifically, the number of autoantibodies detected seems crucial for the prediction of disease, with a relatively low risk associated with positivity for a single autoantibody, increasing to near certainty of developing type 1 diabetes following the appearance of multiple (i.e. two or more) autoantibodies (4-13). Therefore, seroconversion from single to multiple autoantibodies appears to be the hallmark of a 'point of no return' in the type 1 diabetes pathogenetic process, marking the transition from a state of predisposition to a preclinical stage of the disease (3). Associations have been described with younger age and HLA class II genotype (6, 10), but the determinants of this maturation remain largely unknown.

Previous analyses in relatives followed prospectively in the TrialNet Pathway to Prevention (PTP) (formerly Natural History Study) (14,15) have indicated heterogeneity in the development and spreading of islet autoantibody responses, and have also shown that progression from single to multiple autoantibodies is not restricted to early childhood and high risk genotypes (16,17). In light of this evidence and the implications for design of targeted interventions to prevent or delay progression of islet autoimmunity, we have undertaken a more in-depth investigation into the transition from single to multiple autoantibodies, with specific regard to the impact of age in relation to antibody type.

Methods

Study population

Non-diabetic first, second and third degree relatives of people with type 1 diabetes were recruited to the TrialNet PTP (ClinicalTrials.gov identifier: NCT00097292) as previously described (14). All study participants gave informed consent and the Ethics Committee responsible for each clinical site approved the study. Participants were included in this analysis if they had antibodies to the same single islet autoantigen (GADA, IAA or IA-2A) detected on at least two occasions, and antibody results were available from at least one subsequent study visit. All samples were screened for GADA, IAA and IA-2A, and if levels of any of these were above the threshold of positivity, samples were additionally tested for islet cell antibodies (ICA) and ZnT8A. Individuals

with confirmed islet autoantibodies underwent baseline assessment including oral glucose tolerance testing and were followed every 6–12 months in accordance with the PTP study protocol, as previously reported (14). Relatives with the protective HLA DQB1*0602 allele were not included in this analysis.

Assays

GADA, IAA, IA-2A and ZnT8A were measured by radioimmunoassay in the TrialNet Core laboratory at the Barbara Davis Center for Childhood Diabetes (BDC), Denver, CO and ICA by indirect immunofluorescence at the University of Florida, Gainesville, FL, as previously described (18-20).

Statistical analysis

The primary outcome of the analysis was confirmed development of multiple autoantibodies defined as detection on two occasions of at least two of the five islet autoantibodies included in the testing strategy (GADA, IAA, IA-2A, ZnT8A and ICA); this confirmation is based on two consecutive autoantibody tests that are done within one year of each other. The time-to-event was calculated from the date of first detection of a single islet autoantibody to date of first detection of multiple autoantibodies. The risk of developing multiple autoantibodies was assessed by survival analysis using Kaplan–Meier curves. Cox proportional hazards regression models were used for multivariable analysis. Age groups were initially categorized using boundaries based on common definitions of infancy to pre-pubertal, adolescence and adulthood (<8yr; 8-11yr; 12-17yr; ≥18yr); and then refined by recursive partitioning analysis used to identify optimal age breakpoints within each single autoantibody population (21-23). Recursive partitioning is a model-based method used to identify a cutpoint for a marker that best differentiates subjects in relation to an outcome of interest, such as risk of progression to multiple positive autoantibodies.

Results

Of 151,458 relatives screened in the TrialNet PTP between March 1st 2004 and March 31st 2015, 994 were positive for a single autoantibody (GADA, IAA or IA-2A) with normal oral glucose tolerance at baseline and were therefore eligible for inclusion in the analysis, while an additional 276 subjects had abnormal glucose tolerance. Of the 994 positive for a single autoantibody with normal glucose tolerance, 709 (71.3%) had GADA, 236 (23.7%) had IAA and 49 (4.9%) had IA-2A; 59.6% were female.

The median age of the participants was 17.6 years (interquartile range 9.8 - 36.2); 183 (18.4%) were aged less than 8 years, 157 (15.8%) between 8 and 11 years, 169 (17.0%) between 12 and 17 years and 485 (48.8%) 18 years or more.

After a median follow-up of 2.0 years (IQR 0.8-3.8), 141 relatives had developed ≥ 1 additional autoantibodies. Estimated cumulative risk within 5 years was 23% (95% CI 19-27) overall, and did

not vary between autoantibody types (GADA 25% [CI 20-30]; IAA 19% [CI 11-27]; IA-2A 23% [CI 7-38]; p=0.09).

The overall risk of developing additional autoantibodies was inversely related to age (multivariable HR 0.96, 95% CI 0.94-0.99, p=0.005), but heterogeneity between autoantibody type was identified. Table 1 shows the risk of developing multiple autoantibodies within 5 years categorized by age. Relatives with GADA showed a gradual decrease in risk across the four age groups, while relatives with IAA showed a sharp decrease in risk above the age of 8 years. Analysis between age groups was not performed in relatives with IA-2A due to an insufficient number of subjects.

The age distribution of the relatives who developed multiple autoantibodies also differed between those with GADA or IAA (Figure 1). Relatives below 8 years of age represented 71% of the IAA-positive individuals who became multiple autoantibody-positive within 5 years, while the age of GADA-positive individuals who progressed were more evenly distributed throughout childhood and adolescence. Of the relatives who developed one or more additional autoantibodies, 17/24 (71%) with IAA vs 22/99 (22%) with GADA were aged <8 years (p=0.048).

Recursive partitioning analysis confirmed differences in age-related risk between GADA-positive and IAA-positive groups and identified age breakpoints at 14 years in relatives with GADA alone and 4 years in those with IAA alone. The survival time from initial detection of a single islet autoantibody to first detection of multiple antibodies in subgroups categorized by these age breakpoints for GADA-positive relatives is shown in Figure 2A, and for IAA-positive relatives in Figure 2B. Among the GADA-positive relatives, 99 individuals developed additional autoantibodies within 5 years, of whom 59 were aged less than 14 years and 40 were aged ≥14 years (estimated cumulative risk 35.2% [95% CI] (27.9, 43.8) vs 17.7% [95% CI] (12.6, 24.6), respectively; logrank test p<0.001). Among IAA-positive relatives, 24 individuals developed additional autoantibodies within 5 years, of whom 12 were aged less than 4 years and 12 were aged ≥4 years (estimated cumulative risk 73.1% [95% CI] (48.5, 92.6) vs 11.4% [95% CI] (6.3, 20.4), respectively; logrank test, p<0.001).

Discussion

The main finding of this analysis is that the interaction between age and risk of progression from single to multiple autoantibodies differs according to whether the primary autoantigen is GAD or insulin. Specific age breakpoints were very different in those initially positive for IAA compared to those with GADA. This suggests that early interventions targeting single autoantibody-positive individuals at risk for type 1 diabetes may differ in their effectiveness at different ages, depending on whether they have GADA or IAA and provides some guidance as to what age groups to target.

Previous analyses in relatives followed prospectively in the TrialNet PTP (14,15) found that, in those positive for a single autoantibody, the risk of progression to multiple antibodies was 22% within 5 years and to type 1 diabetes was 6% (16). As confirmed in the present analysis, overall risk of development of multiple autoantibodies was independent of antibody type, inversely related to age, and associated with high and intermediate risk HLA Class II genotypes and high

GADA titers (16). In addition, progression appeared not to be influenced by initial body mass index or other metabolic variables (24). More recently, recursive partitioning analysis demonstrated that age and GADA titer taken together were helpful in stratifying the overall risk of progression from single to multiple autoantibodies (17).

These additional analyses focus on evaluation of the time course of progression among single autoantibody-positive subjects, depending whether the first autoantibodies detected were GADA or IAA. These demonstrate marked differences between the initial IAA and GADA groups. Although risk was inversely related to age in both groups, the relationships were not the same; while risk in GADA-positive relatives decreased gradually up to age 18 years, in IAA-positive relatives risk was concentrated in younger children with high risk of progression up to age 8 years, and a sharp decline thereafter. This pattern was confirmed by recursive partitioning analysis which showed significant differences in risk and identified different age breakpoints for IAA (4 years) and for GADA (14 years).

Based on these observations, spread of autoimmunity from insulin to other islet antigens seems largely limited to early childhood, while autoimmune responses initially directed against GAD can mature over a longer period of time, including the whole of adolescence. It will be interesting to see whether this pattern is confirmed in studies of cellular immune responses.

These findings are relevant to the design of prevention trials targeting the early phases of type 1 diabetes-associated autoimmunity. In individuals with IAA alone, intervention should be considered only below the age of 8 years, with the highest priority given to individuals below 4 years of age; conversely, in individuals with GADA alone, intervention seems justified up to the age of 18 years, with the highest potential below 14 years. Although there is no sharp decline after age 18, the overall risk of developing additional antibodies is relatively low in GADA-positive adults (16% within 5 years) and we suggest that issues of power and the sample size required should be carefully evaluated before including them in type 1 diabetes prevention trials.

A particular strength of this study is the size of the cohort, screened and followed up every 6-12 months according to a standard protocol (14). One limitation is that, in contrast with studies following infants from birth, the time at which seroconversion has occurred is unknown when relatives are found to be positive for an autoantibody in the TrialNet PTP. This may potentially lead to underestimation of the duration of single autoantibody positivity but, since the focus of this study is the time to further seroconversion to multiple autoantibodies, the time of initial seroconversion is less relevant in this context. As in other TrialNet studies, we have included appearance of ICA in the definition of development of multiple autoantibodies because, although GADA and I-2A contribute to ICA staining (25,26), there is not complete overlap and previous analyses in the PTP cohort have demonstrated that ICA are associated with additional risk (20).

Although the determinants of islet autoimmunity underlying type 1 diabetes remain unknown, accumulating evidence suggests that it develops through multistep involvement of several pathogenetic pathways (27). Accordingly, interventions at an earlier stage might rely on simpler approaches, such as antigen based therapies, while at a later stage, a more complex approach,

based for instance on combination therapies, is likely to be necessary to halt or delay the progression of the disease process (28). Based on our current ability to predict type 1 diabetes, single autoantibody positivity is the earliest detectable sign of the ongoing autoimmune process, when the chances of a successful intervention could be greatest. The evidence of heterogeneity in the progression of islet autoimmunity in relatives of the TrialNet PTP study, the largest study cohort ever screened and followed up, calls for different therapeutic approaches in single autoantibody positive individuals with IAA or GADA, with the highest potential for success in interventions designed for early childhood in IAA-positive and no later than adolescence in GADA-positive individuals.

Author contribution

All authors were members of the TrialNet Study Group and contributed to the data used in this article. EB and PJB wrote the manuscript. PJB and DCB designed and conducted the statistical analysis. All authors contributed to discussion, reviewed/edited the manuscript and gave final approval for the paper to be published. SG is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1) Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2014;383:69-82

2) Bonifacio E. Predicting type 1 diabetes using biomarkers. Diabetes Care 2015;38:989-996

3) Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark Å, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964-1974

4) Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473-2479

5) Orban T, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G; Diabetes Prevention Trial-Type 1 Study Group. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 2009;32:2269-2274

6) Ziegler AG, Bonifacio E; BABYDIAB-BABYDIET Study Group. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. Diabetologia 2012;55:1937-1943

7) Steck AK, Johnson K, Barriga KJ, Miao D, Yu L, Hutton JC, Eisenbarth GS, Rewers MJ. Age of islet autoantibody appearance and mean levels of insulin, but not GAD or IA-2 autoantibodies, predict age of diagnosis of type 1 diabetes: diabetes autoimmunity study in the young. Diabetes Care 2011;34:1397-1399

8) Parikka V, Näntö-Salonen K, Saarinen M, Simell T, Ilonen J, Hyöty H, Veijola R, Knip M, Simell O. Early seroconversion and rapidly increasing autoantibody concentrations predict

prepubertal manifestation of type 1 diabetes in children at genetic risk. Diabetologia 2012;55:1926-1936

9) Steck AK, Vehik K, Bonifacio E, Lernmark A, Ziegler AG, Hagopian WA, She J, Simell O, Akolkar B, Krischer J, Schatz D, Rewers MJ; TEDDY Study Group. Predictors of Progression From the Appearance of Islet Autoantibodies to Early Childhood Diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes Care 2015;38:808-813

10) Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E; TEDDY Study Group. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980-987

11) Achenbach P, Hummel M, Thümer L, Boerschmann H, Höfelmann D, Ziegler AG. Characteristics of rapid vs slow progression to type 1 diabetes in multiple islet autoantibody-positive children. Diabetologia. 2013;56:1615-1622

12) Chmiel R, Giannopoulou EZ, Winkler C, Achenbach P, Ziegler AG, Bonifacio E. Progression from single to multiple islet autoantibodies often occurs soon after seroconversion: implications for early screening. Diabetologia. 2015;58:411-3

13) Steck AK, Dong F, Waugh K, Frohnert BI, Yu L, Norris JM, Rewers MJ. Predictors of slow progression to diabetes in children with multiple islet autoantibodies. J Autoimmun. 2016;72:113-117

14) Mahon JL, Sosenko JM, Rafkin-Mervis L, Krause-Steinrauf H, Lachin JM, Thompson C, Bingley PJ, Bonifacio E, Palmer JP, Eisenbarth GS, Wolfsdorf J, Skyler JS; TrialNet Natural History Committee; Type 1 Diabetes TrialNet Study Group. The TrialNet Natural History Study of the Development of Type 1 Diabetes: objectives, design, and initial results. Pediatr Diabetes 2009;10:97-104

15) Vehik K, Beam CA, Mahon JL, Schatz DA, Haller MJ, Sosenko JM, Skyler JS, Krischer JP; TrialNet Natural History Study Group. Development of autoantibodies in the TrialNet Natural History Study. Diabetes Care. 2011;34:1897-1901

16) Bingley PJ, Boulware DC, Krischer JP; Type 1 Diabetes TrialNet Study Group. The implications of autoantibodies to a single islet antigen in relatives with normal glucose tolerance: development of other autoantibodies and progression to type 1 diabetes. Diabetologia 2016;59:542-549

17) Xu P, Krischer JP. Prognostic Classification Factors Associated With Development of Multiple Autoantibodies, Dysglycemia, and Type 1 Diabetes-A Recursive Partitioning Analysis. Diabetes Care 2016;39:1036-1044

18) Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth GS. Antiislet autoantibodies develop sequentially rather than simultaneously. J Clin Endocrinol Metab 1996;81:4264–4267

19) Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, Adler K, Ziegler AG, Mueller PW, Schatz DA, Krischer JP, Steffes MW, Akolkar B. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of

diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab. 2010;95:3360-3367

20) Yu L, Boulware DC, Beam CA, Hutton JC, Wenzlau JM, Greenbaum CJ, Bingley PJ, Krischer JP, Sosenko JM, Skyler JS, Eisenbarth GS, Mahon JL; Type 1 Diabetes TrialNet Study Group. Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. Diabetes Care 2012;35:1213–1218

21) Ciampi IA, Thiffault J. Recursive partition and amalgamation (RECPAM) for censored survival data: criteria for tree selection. Stat Softw Newsletter 1988;14:78–81

22) Gordon L, Olshen RA. Tree-structured survival analysis. Cancer Treat Rep 1985;69:1065–1069

23) Breiman L, Friedman JH, Olshen RA, Stone CJ. Classification and Regression Trees. Belmont, CA, Wadsworth International Group, 1984

24) Meah FA, DiMeglio LA, Greenbaum CJ, Blum JS, Sosenko JM, Pugliese A, Geyer S, Xu P, Evans-Molina C; Type 1 Diabetes TrialNet Study Group. The relationship between BMI and insulin resistance and progression from single to multiple autoantibody positivity and type 1 diabetes among TrialNet Pathway to Prevention participants. Diabetologia 2016;59:1186-1195

25) Richter W, Seissler J, Northemann W, Wolfahrt S, Meinck HM, Scherbaum WA. Cytoplasmic islet cell antibodies recognize distinct islet antigens in IDDM but not in stiff man syndrome. Diabetes. 1993;42:1642-1648.

26) Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. J Immunol. 1995;155:5419-1526

27) Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 2010;464:1293-1300

28) Skyler JS. Prevention and reversal of type 1 diabetes--past challenges and future opportunities. Diabetes Care 2015;38:997-1007

Legend to table and figures

Table 1. Risk of developing multiple autoantibodies within 5 yr in single autoantibody-positive relatives categorized by age (95% CI).

Figure 1: GADA-positive (panel A, n=99) and IAA-positive participants (panel B, n=24) who developed additional autoantibodies within 5 years subdivided by age group. Among the relatives who progressed, 17/24 (71%) with IAA vs. 27/99 (27%) GADA were aged <8 years ($p<0.05$).

Figure 2.- Time from initial detection of: **A)** single GADA to first detection of multiple antibodies in relatives aged <14 years (black line) and ≥14 years (grey line); and **B)** of single IAA to first detection of multiple antibodies in relatives aged <4 years (black line) and ≥4 years (grey line)

347 **Table 1** - Risk of developing multiple autoantibodies within 5 yr in single autoantibody-positive
348 relatives categorized by age (95% CI)

349

Antibody type	**Age at entry (n)**				**Overall**
	<8 yr	8-11 yr	12-17yr	>18 yr	
GADA (n)	107	113	117	372	709
- Risk	35% (24-46)	38% (24-51)	28% (14-41)	16% (10-22)	25% (20-30)
IAA (n)	69	33	43	91	236
- Risk	37% (22-52)	4% (0-11)	13% (0-34)	13% (0-25)	19% (11-27)
IA-2A (n)	7	11	9	22	49
- Risk	*Insufficient number of subjects to assess*				23% (7-38)

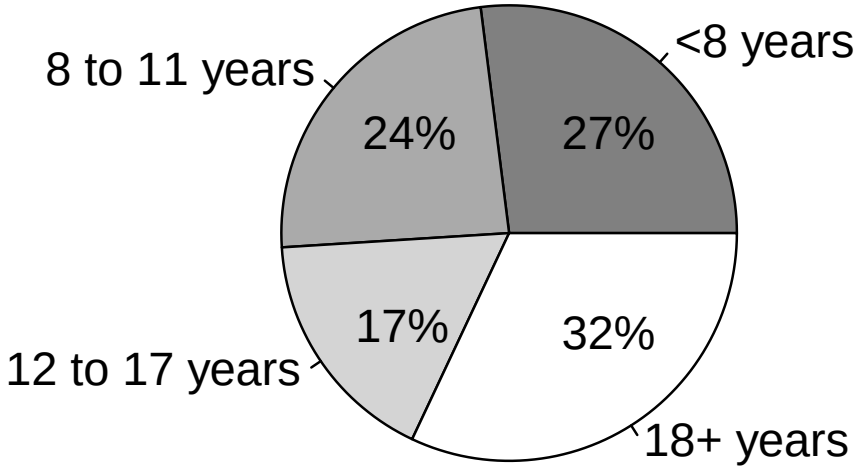
350

351

Table 1 - Risk of developing multiple Ab within 5 yr in single Ab-positive relatives categorized by age (95% CI)

Ab type	**Age at entry (n)**				**Overall**
	<8 yr	8-11 yr	12-17yr	>18 yr	
GADA (n)	107	113	117	372	709
- Risk	35% (24-46)	38% (24-51)	28% (14-41)	16% (10-22)	25% (20-30)
IAA (n)	69	33	43	91	236
- Risk	37% (22-52)	4% (0-11)	13% (0-34)	13% (0-25)	19% (11-27)
IA-2A (n)	7	11	9	22	49
- Risk	*Insufficient number of subjects to assess*				23% (7-38)

A



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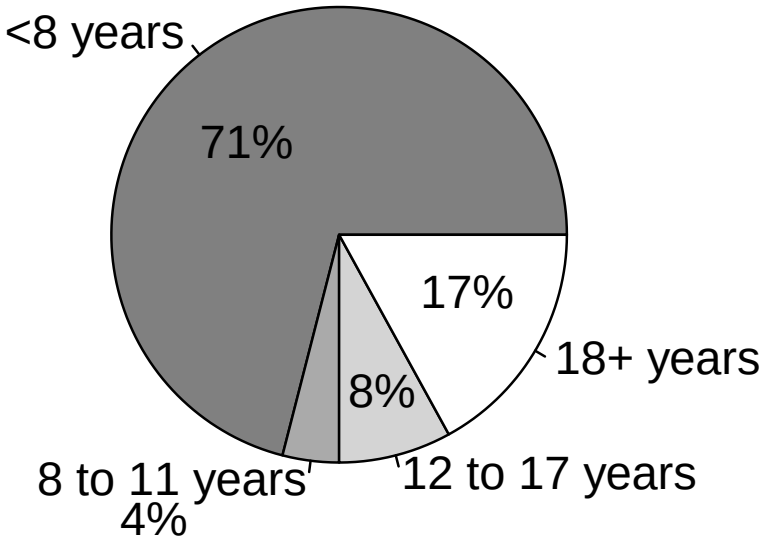

Figure

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